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Volume 156, number 2 FEBS 0467 June 1983

A non-micellar mode of cholesterol transport in human bile

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Received 24 April 1983

Quasi-elastic light scattering (QELS) was used to measure particle size in fresh human hepatic bile of 14 subjects. Particles with an approximate diameter of 700 Å were found in all biles. The particles were almost unchanged after the bile salt concentration was reduced to 0.06 mM by dilution or dialysis against 150 mM NaCl. During dialysis bile salts were removed, while cholesterol and phospholipids remained in solution apparently in the large particles–vesicles. These experiments suggest the presence of a novel, bile salt-independent, mode of cholesterol transport in saturated human bile.

Cholesterol *Bile salt* *Phospholipid* *Light scattering, quasi-elastic*

1. INTRODUCTION

The widely accepted model of cholesterol transport in bile assumes its transport in mixed micelles of bile salts and phospholipids [1,2]. The triangular phase diagram that shows the aggregational state of lipids, and is the backbone of the micellar theory [2], was based on experiments using model systems of the three biliary lipids in water. In these model systems the amount of lipids participating in the formation of micelles is determined by interaction between free molecules. In the heterogeneous native bile the situation may be more complex, since part of the lipid molecules may be bound, and the inferences based on model systems may not be necessarily correct. The presence of micelles and the amount of cholesterol transported by them were not directly demonstrated in bile.

Quasi-elastic light scattering (QELS) was used to characterize populations of particles such as bile salt micelles in water [3,4]. In this study we have used QELS in an attempt to measure lipid particles in native human bile [5]. Large 700 Å particles were found all hepatic biles. These particles apparently contained cholesterol and phospholipids and persisted in the absence of bile salts.

2. MATERIALS AND METHODS

Hepatic bile was obtained from patients after choledochotomy with an in-dwelling T-tube. Fresh bile was kept at ambient temperature (20–30°C) and measurements were performed within 1–6 h except for one subject whose bile was examined a few minutes after secretion. For lipid analysis aliquots were kept at −20°C. Dilutions of bile were done with sterile 0.15 M NaCl, and dialysis in a Thomas dialysis tubing with a cut-off limit of M_r 12000. Samples of 2 ml were dialyzed against 1 liter 0.15 M NaCl for 18 h at room temperature. Prior to the QELS measurements all samples were centrifuged for 2 min at 85000 × *g* in an airfuge with an A-100/18 rotor (Beckman) in cellulose nitrate 5 × 20 mm disposable tubes (Beckman) for removal of dust and cellular debris. The sample tube was immersed in water in the center of a cylindrical glass cell (3 cm diam.) that served as a water bath. The temperature of the water bath was kept constant at 37 ± <0.1°C and was monitored by a precision thermistor (YSI) connected to a digital multimeter (Fluke, model 8600A). The light source was a He–Ne laser (Spectra-Physics, model 125) with 60 mW power maximum at wavelength of 632.8 nm. The light beam was focused on the sam-

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ple cell by lens. The scattered light at 90° was focused on the photomultiplier (Pacific Precision Instruments model 3262/AD6 RCA 8850) by a lens. A vertical slit (width 0.15 mm) and a pinhole slit (diam. 0.1 mm) were installed between the sample cell and the lens to eliminate stray light. The photomultiplifer was used at 1700 V; its output was collected by a correlator with 64 channels and 8 delayed channels (Langley-Ford Instruments model 1096). The correlator was used in autocorrelation mode. The cumulants data analysis was supplied with the correlator. The Z-average translational diffusion coefficient $D_z = <\Gamma>/K^2$ was calculated from the average decay rate and the scattering vector $K = (4\pi n/\lambda_o) \sin(\theta/2)$ where n is the refractive index of the solvent, λ_o is the wavelength in vacuo and θ is the gyration angle. The equivalent hydrodynamic radius ($\bar{R}_h$) was calculated from the Stokes–Einstein equation: $\bar{R}_h = kT/6\pi\eta D$ where k is the Boltzman constant, T is the absolute temperature and η is the viscosity of the solvent.

Lipid analysis was performed on all bile specimens after total lipid extraction by chloroform and methanol, 3:1 (v/v) [6]. In the chloroform phase, cholesterol was determined after KOH hydrolysis [7] and phospholipids after perchloric acid oxidation by phosphate determination [8]. In the methanolic phase, bile salts were determined enzymatically by 3α-hydroxysteroid-dehydrogenase [9].

3. RESULTS

The mean ± SD translational diffusion coefficient (D) of particles in fresh human hepatic bile as measured by QELS was $1 \times 10^{-7} \pm 0.1 \times 10^{-7}$ cm^2/s (n = 14). The mean particle diameter was 680 ± 80 Å. The coefficient of variation of particle size in individual biles was 2.6% and the variation between biles was 12%. Using a fit of cumulants to the decaying exponentials of the scattered light, the second cumulant μ_2/Γ^2 for most of the biles was under 0.1. Calculation of the hydrodynamic radii from the Stokes–Einstein equation assumed the viscosity (η) and refractive index (n) of the native biles to equal those of 150 mM NaCl. To check the validity of this calculation, stepwise dilutions with 150 mM NaCl were performed with concomitant QELS measurements (table 1). Dilutions up to 1:30 produced no consistent change in particle size. The maximal deviation from the original measurement was ~20% when >97% of the bile was replaced by the salt solution.

To further validate the above measurements and also to determine whether the particles were aggregates of mixed micelles, fresh hepatic bile was dialyzed against 150 mM NaCl. The soluble small-M_r components in the dialyzed biles (including most of the bile salts) were removed and the solvent became similar to 150 mM NaCl. The particles in the dialyzed biles remained intact on QELS examination and had a diameter of 720 ± 100 Å (table 2). The maximal change in particle size after dialysis was <30%. There was no significant change in the intensity of light scattered by the particles before and after dialysis. This provides additional evidence that the number of particles remained similar following dialysis.

Table 1

Particle diameter (Å), calculated from QELS measurements, following stepwise dilutions

		Dilution				
		1:1	1:3	1:6	1:15	1:30
Bile 1	605	550	557	571	654	
Bile 2	699	709	687	861		
Bile 3	653	547	580	529	609	826
Bile 4	797	756	753	675	678	611

Fresh hepatic biles were diluted with 150 mM NaCl

Table 2

Particle size and lipid composition following dialysis

	Before dialysis				After dialysis			
Bile	Diam. Å	BS mM	PL mM	Ch mM	Diam. Å	BS mM	PL mM	Ch mM
1	605	14.6	15	5.5	517	0.5	12.5	5.1
2	728	3.9	1.2	1.2	525	0.06	1.8	1.3
3	816	11.7	6.9	1.7	973	4.8	6.4	1.4

Fresh hepatic bile (2 ml) was dialyzed for 18 h at room temperature against 1 liter of 150 mM NaCl. Particle diameter was calculated from QELS measurements. Bile salts (BS), phospholipid (PL) and cholesterol (Ch) were analyzed

Table 3

Particle size and lipid composition of biles

Bile	Diam. (Å)	BS (mM)	PL (mM)	Ch (mM)	Total lipids (g/dl)
1	605	14.6	15	5.5	2.3
2	728	3.9	1.2	1.2	0.3
3	816	11.7	6.9	1.7	1.2
4	699	1.8	0.7	0.7	0.2
5	797	17.9	7.3	3.9	1.6
6	653	7.6	3.4	2.6	0.6
7	575	10.6	7.1	5.1	1.3
8	628	24.8	0.7	0.7	1.6
9	667	4.6	6.1	3.9	0.9
10	677	25.7	5.2	1.7	1.2
11	586	5.7	0.5	0.9	0.3
12	550	5.8	2.5	1.8	0.5
13	743	14.4	6.7	1.6	1.3
14	727	18.1	6.6	1.7	1.5
Mean ± SD	675 ± 82	11.9 ± 7.6	5 ± 3.9	2.4 ± 1.6	1.1 ± 0.6

The dilution and dialysis experiments showed that the particles exist independently of bile salts. At the highest dilution (1:30) bile salt concentration was 0.06 mM which is far below the critical micellar concentration (~2–5 mM for most bile salts). In two of the dialysis experiments the final bile salt concentration in the dialyzed bile was <0.5 mM, while $>80\%$ of the cholesterol and phospholipids remained in the dialysis bag. To determine whether the cholesterol in the dialyzed bile remained in solution or had precipitated, the dialyzed bile was examined by polarization microscopy and chemical analysis was performed. Polarized light microscopy did not reveal any cholesterol crystals. Following centrifugation chemical analysis did not detect any cholesterol in the pellet; cholesterol concentration in the supernatant solution remained unchanged. The supernatant solution was examined by QELS and there was no indication for the formation of cholesterol crystals. The particles measured by QELS in the dialyzed biles were even more homogeneous and had a smaller μ_2/Γ^2.

The lipid composition of the 14 biles is listed in table 3 and shown in fig.1. All biles were saturated or supersaturated with cholesterol.

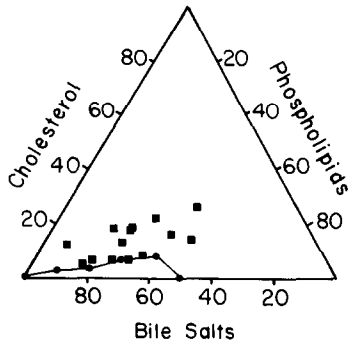

Fig.1. Lipid composition (■) of biles in mol%; mean lipid concentration of biles was 1.1 g/dl; micellar solubility limits (●—●) for 1.25 g lipid/dl at 37°C [2].

4. DISCUSSION

The consistent presence of large cholesterol-containing particles, had not been described in human bile. We have also performed preliminary studies of human bile by electron microscopy using freeze fracture and deep etching. Particles of ~700 Å were found having the appearance of vesicles. The presence of these particles–vesicles was thus confirmed by two independent methods.

The particle size measured by QELS showed considerable uniformity in each individual bile and variations in size between biles of different subjects. It is uncertain whether these variations reflect differences in the physical characteristics of various biles or changes in the composition and the actual size of the particles. Large 400 and 1000 Å particles were occasionally noted in model solutions [10] and animal biles [11]. They were thought to represent a minor population of biliary particles, 0.1–1% and to carry excess cholesterol or to be protein or pigment aggregates [10].

The dialysis experiments in this study demonstrate that cholesterol can remain in solution apparently within the particles–vesicles in the virtual absence of bile salts. Under these experimental conditions, the particles–vesicles were able to carry >80% of the cholesterol present in the supersaturated hepatic bile. Other investigators have also noted that during dialysis of bile cholesterol and phospholipids remained in the bag while bile salts were removed to a variable extent [12,13]. The dilution experiments also confirmed that the particles–vesicles persisted at minimal bile salt concentrations, far below the critical micellar concentration.

In these light scattering experiments it could not be determined whether micelles are present in fresh hepatic bile. The high scattering intensity of the large particles would obscure the light scattered by 20–60 Å micelles if present. It cannot be determined at present what proportion of the biliary cholesterol is transported originally within the particles–vesicles. Is there an equilibrium between the cholesterol transported by vesicles and micelles? Are there differences in this respect between undersaturated and supersaturated biles? These problems are being examined.

This work has demonstrated a novel, bile salt-independent, form of cholesterol transport in human bile. The current concepts of cholesterol solubilization and precipitation in human bile may have to be reevaluated.

ACKNOWLEDGEMENTS

The light scattering experiments were performed in the Department of Polymer Research, Weizmann Institute of Science (Rehovot). We are especially indebted to Professor Zvi Kam and Dr Nina Borochov for their valuable help and guidance.

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